

A Hybrid Hoare Logic for Gene Network Models

Jonathan Behaegel, Jean-Paul Comet, and Maxime Folschette *

University Nice Sophia Antipolis and I3S laboratory, UMR CNRS 7271
CS 40121, 06903 Sophia Antipolis CEDEX, France
behaegel@i3s.unice.fr, {comet,maxime.folschette}@unice.fr

Abstract. The main difficulty when modelling gene networks is the identification of the parameters that govern their dynamics. It is particularly difficult for models in which time is continuous: parameters have real values which cannot be enumerated. The widespread idea is to infer new constraints that reduce the range of possible values. Here we present a new work based on a particular class of Hybrid automata (inspired by Thomas discrete models) where discrete parameters are replaced by signed celerities. We propose a new approach involving Hoare logic and weakest precondition calculus (a la Dijkstra) that generates constraints on the parameter values. Indeed, once proper specifications are extracted from biological traces with duration information (found in the literature or biological experiments), they play a role similar to imperative programs in the classical Hoare logic. We illustrate our hybrid Hoare logic on a small model controlling the *lacI* repressor of the lactose operon.

Keywords: gene networks, hybrid automata, constraint synthesis, Hoare logic, weakest precondition

1 Introduction

Regulatory networks are models based on gene regulation in the aim of representing their functioning. They are “on/off” switching systems of genes whose role is to control when each gene acts and when its corresponding protein is synthesised. When a gene switches on/off, its protein is synthesised or degraded leading to a change of gene regulations. The main difficulty of such networks is the identification of parameters, whatever the modelling framework (differential, qualitative or stochastic models). These parameters govern the dynamics of the system, and constraints should be identified at least to narrow the set of suitable parameters. We need to accurately identify these parameters especially when time plays an essential role like in the the coupling between the cell cycle and the circadian clock.

Previous studies used René Thomas’ discrete modelling framework [13] to determine the dynamics of such systems [6]. This modelling is based on discretisation of concentration spaces, so each threshold separates concentration areas

* alphabetically ordered authors

where the regulations are identical (the regulations are supposed to be sharp sigmoid).

The hybrid framework we use in this paper describes concentration levels in a qualitative way as in Thomas' discrete modelling, but it also takes into account temporal information represented by evolution speeds (called celerities). The time spent inside each discrete state can be approximated through biological data, thus increasing the constraints on parameters and improving the exactness of the model [7]. Such a hybrid model of the cell cycle was created [3] and, using a suitable set of parameters, we produced simulations in accordance with the classical known behaviour of the cell cycle.

In this paper we use Hoare logic in order to establish constraints on the celerities. Similar approaches were already developed for Thomas' discrete approach [5,4] and extensions on more generic hybrid automata have been proposed [11,10,14]. Hoare logic relies on so-called Hoare triples of the form $\{Pre\} p \{Post\}$ (where Pre and $Post$ are formulas and p is a path) [9] which mean that, starting from a state satisfying the precondition Pre and crossing the path p , the trajectory reaches a point where the postcondition $Post$ is satisfied. However, in this work, we mainly focus on the computation of the weakest precondition Pre for a given path p and postcondition $Post$ [8].

So, using biological data, represented into a Hoare triple, we are able to establish new constraints on parameters allowing the model to behave as the observed traces. The description of the observed traces essentially consists in depicting the correct order of the events (i.e., the order of crossings of thresholds). According to the situation, we can also use other information, such as the saturation or complete degradation of a protein which lead to additional constraints.

We illustrate our approach on a genetic construct in *Escherichia Coli* composed of the lactose operon *lacI* and some elements of the system *Ntr* [2]. The *glnA* promoter of system *Ntr* is modified by adding the operator site of the *lacI* repressor (inhibition). Similarly the *glnK* promoter of *Ntr* is fused to the *lacI* gene (activation). These alterations lead to an oscillatory behaviour in *E. Coli*. We deduce with our weakest precondition calculus the constraints on celerities mandatory to observe oscillation.

The paper is organised as follows. We first define the hybrid modelling framework based on Thomas' discrete one (Sec. 2). Then Sec. 3 is focused on the hybrid Hoare logic. Section 4 details one step of the parameter identification on the biological example of the *lacI* repressor interacting with the *Ntr* system. Finally, we discuss the limits of this approach in Sec. 5.

2 Hybrid Modelling Framework

A gene network is visualised as a labelled directed graph (interaction graph) in which vertices are either variables (within circles) or multiplexes (within rectangles), see Fig. 1. Variables abstract genes and their products, and multiplexes contain propositional formulas that encode situations in which a variable or a group of variables (inputs of multiplexes, dashed arrows) influences the evolu-

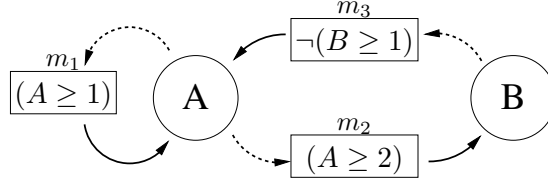


Fig. 1. The gene network controlling the *lacI* repressor regulation of the lactose operon in *E. Coli*. See Sec. 4 for more details.

tion of some other variables (output of multiplexes, plain arrows). A multiplex can encode the formation of molecular complexes, phosphorylation by a protein, competition of entities for activation of a promoter, etc. Definition 1 gives the formal details of a gene network.

Definition 1 (Hybrid gene regulatory network). A hybrid gene regulatory network (GRN for short) is a tuple $R = (V, M, E, C)$ where:

- V is a set whose elements are called variables of the network. Each variable $v \in V$ is associated with a boundary $b_v \in \mathbb{N}^*$.
- M is a set whose elements are called multiplexes. With each multiplex $m \in M$ is associated a formula φ_m belonging to the language \mathcal{L} inductively defined by:
 - If $v \in V$ and $n \in \mathbb{N}$ such that $1 \leq n \leq b_v$, then $v \geq n$ is an atom of \mathcal{L} ;
 - If φ and ψ are two formulas of \mathcal{L} , then $\neg\varphi$, $(\varphi \vee \psi)$, $(\varphi \wedge \psi)$ and $(\varphi \Rightarrow \psi)$ also belong to \mathcal{L} .
- E is a set of edges of the form $(m \rightarrow v) \in M \times V$.
- $C = \{C_{v,\omega,n}\}$ is a family of real numbers indexed by the tuple (v, ω, n) where v, ω and n verify the three following conditions:
 1. $v \in V$,
 2. ω is a subset of $R^-(v)$ where $R^-(v) = \{m \mid (m \rightarrow v) \in E\}$, that is, ω is a set of predecessors of v ,
 3. n is an integer such that $0 \leq n \leq b_v$. $C_{v,\omega,n}$ is called the celerity of v for ω at the level n .

Moreover, celerities are constrained. For each $v \in V$ and for each $\omega \subset R^-(v)$:

- either all celerities $C_{v,\omega,n}$ with $0 \leq n \leq b_v$ have the same nonzero sign,
- or there exists n_0 such that $C_{v,\omega,n_0} = 0$ and for all n such that $0 \leq n < n_0$, we have $\text{sgn}(C_{v,\omega,n}) = 1$ and for all n such that $n_0 < n \leq b_v$, we have $\text{sgn}(C_{v,\omega,n}) = -1$ where the function sgn is the classical sign function.

Let us remark that the dashed arrows of Fig. 1 are not present in the previous definition. When representing a gene network, it is convenient to visualise the variables contributing to a particular multiplex, but from a formal point of view, this information is coded into the formula of the considered multiplex.

In the remainder of this section, we focus on the dynamics of such a gene network. Definition 2 introduces the hybrid states whereas Def. 3 explains the crucial notion of resources of a variable at a particular state.

Definition 2 (State of a GRN). Let $R = (V, M, E, \mathcal{C})$ be a GRN. A hybrid state of R is a tuple $h = (\eta, \pi)$ where

- η is a function from V to \mathbb{N} such that for all $v \in V$, $0 \leq \eta(v) \leq b_v$;
- π is a function from V to the interval $[0, 1]$ of real numbers.

η is called the discrete state of h whereas π is called its fractional part. For simplicity, we note in the sequel $\eta_v = \eta(v)$ and $\pi_v = \pi(v)$. We denote S the set of hybrid states of R . When there is no ambiguity, we often use η and π without explicitly mentioning h .

Figure 2-Centre illustrates an example of hybrid state. The tuple of all fractional parts represents coordinates inside the current qualitative state.

Definition 3 (Resources). Let $R = (V, M, E, \mathcal{C})$ be a GRN and let $v \in V$. The satisfaction relation $h \models \varphi$, where $h = (\eta, \pi)$ is a hybrid state of R and φ a formula of \mathcal{L} , is inductively defined by:

- If φ is the atom $v \geq n$ with $n \in \llbracket 1, b_v \rrbracket$, then $h \models \varphi$ iff $\eta_v \geq n$;
- If φ is of the form $\neg\psi$, then $h \models \varphi$ iff $h \not\models \psi$;
- If φ is of the form $\psi_1 \vee \psi_2$, then $h \models \varphi$ iff $h \models \psi_1$ or $h \models \psi_2$ and we proceed similarly for the other connectives.

The set of resources of a variable v for a state h is defined by: $\rho(h, v) = \{m \in R^-(v) \mid h \models \varphi_m\}$, that is, the multiplexes predecessors of v whose formula is satisfied. Thus, ω is the subset of resources of v iff the formula Φ_v^ω is true:

$$\Phi_v^\omega \equiv \left(\bigwedge_{m \in \omega} \varphi_m \right) \wedge \left(\bigwedge_{m \in R^{-1}(v) \setminus \omega} \neg \varphi_m \right). \quad (1)$$

We note that the evaluation of formula Φ_v^ω given in (1) requires only the valuation of the discrete state: all hybrid states having the same discrete part have the same resources. This illustrates the fact that inside a discrete state, the dynamics is controlled in the same manner, thus the celerity is the same: the one associated with the current discrete state and with the current resources $C_{v, \rho(v, \eta), \eta_v}$. From this celerity, and given a particular hybrid state, one can compute the touch delay of each variable, which measures the time necessary for the variable to reach a border of the discrete state.

Definition 4 (Touch delay). Let $R = (V, M, E, \mathcal{C})$ be a GRN, v be a variable of V and $h = (\eta, \pi)$ be a hybrid state. We note $\delta_h(v)$ the touch delay of v in h for reaching the border of the discrete state. More precisely, δ_h is the function from V to $\mathbb{R}^+ \cup \{+\infty\}$ defined by:

- If $C_{v, \rho(v, \eta), \eta_v} = 0$ then $\delta_h(v) = +\infty$;
- If $C_{v, \rho(v, \eta), \eta_v} > 0$ then $\delta_h(v) = \frac{1 - \pi_v}{C_{v, \rho(v, \eta), \eta_v}}$;
- If $C_{v, \rho(v, \eta), \eta_v} < 0$ then $\delta_h(v) = \frac{-\pi_v}{C_{v, \rho(v, \eta), \eta_v}}$.

Unfortunately, being at a border of the discrete state is not sufficient to go beyond the frontier: there may be no other qualitative level beyond the border (we call such a border an external wall) or the celerity in the neighbouring state may be of the opposite sign (internal wall), as given in Def. 5.

Definition 5 (External and internal walls). *Let $R = (V, M, E, C)$ be a GRN, let $v \in V$ be a variable and $h = (\eta, \pi)$ a hybrid state.*

1. v is said to potentially meet an external wall if:

$$((C_{v,\rho(v,\eta),\eta_v} < 0) \wedge (\eta_v = 0)) \vee ((C_{v,\rho(v,\eta),\eta_v} > 0) \wedge (\eta_v = b_v)) .$$

2. Let $h' = (\eta', \pi')$ be another hybrid state s.t. $\eta'_v = \eta_v + \text{sgn}(C_{v,\rho(v,\eta),\eta_v})$ and $\eta'_u = \eta_u$ for all $u \neq v$. Variable v is said to potentially meet an internal wall if:

$$\text{sgn}(C_{v,\rho(v,\eta),\eta_v}) \times \text{sgn}(C_{v,\rho(v,\eta'),\eta'_v}) = -1 .$$

We note $sv(h)$ the set of sliding variables that will potentially meet an internal or external wall in the qualitative state of h .

We note that for all $v \in sv(h)$, if, in addition, $\delta_h(v) = 0$, then v is located on the said internal wall or external wall. In this case, its fractional part cannot evolve anymore in the current qualitative state (see Fig. 2-Right where variable B reaches an external wall). We introduce the notion of knocking variables in Def. 6 which are the first variables able to change their discrete levels.

Definition 6 (Knocking variables). *Let $R = (V, M, E, C)$ be a GRN and $h = (\eta, \pi)$ be a hybrid state. The set of knocking variables is defined by:*

$$\text{first}(h) = \{v \in V \setminus sv(h) \mid \delta_h(v) \neq +\infty \wedge \forall u \in V \setminus sv(h), \delta_h(u) \geq \delta_h(v)\} .$$

Moreover, δ_h^{first} denotes the time spent in the qualitative state of h when starting from h : for any $x \in \text{first}(h)$, $\delta_h^{\text{first}} = \delta_h(x)$.

The set $\text{first}(h)$ represents the set of variables whose qualitative coordinate can change first. If the variable is on an external or internal wall, it cannot evolve as long as other variables do not change. Similarly, if the celerity of v in the current state is null, its qualitative value cannot change because of an infinite touch delay.

Figure 2 illustrates several evolutions of a gene network. From a particular hybrid state P_0 , the dynamics alternates continuous transitions (within the discrete state) and discrete transitions (when changing the discrete state). When the trajectory reaches an external or internal wall (see Right part of the figure) the variable slides along the wall only if the celerity of some other variable can drive the trajectory in such a direction. This description leads to Def. 7.

Definition 7 (Hybrid state space). *Let $R = (V, M, E, C)$ be a GRN, we note $\mathcal{R} = (S, T)$ the hybrid state space of R where S is the set of hybrid states and T is the set of transitions: there exists a transition from state $h' = (\eta', \pi')$ to state $h = (\eta, \pi)$ iff there exists a variable $v \in \text{first}(h')$ such that:*

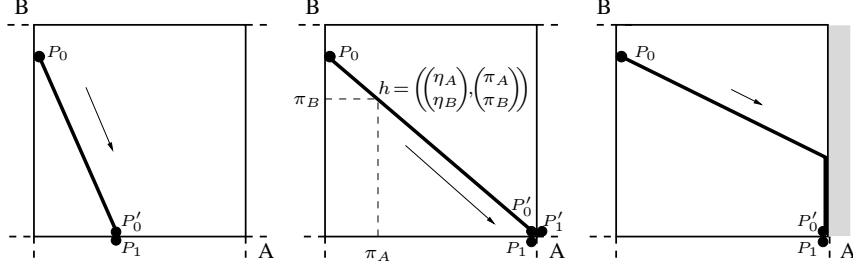


Fig. 2. Continuous transitions. Inside each state, a continuous transition ($P_0 \rightarrow P'_0$) goes from the initial point P_0 to the unique point P'_0 from which a discrete transition takes place ($P'_0 \rightarrow P_1$). **Left:** The celerity vector allows, without sliding mode, the trajectory to directly reach a border which is crossed. **Center:** From P'_0 two possible discrete transitions can occur: $P'_0 \rightarrow P_1$ or $P'_0 \rightarrow P'_1$. Moreover (π_A, π_B) corresponds to the fractionnal coordinates of a hybrid state h along the path. **Right:** The grey zone depicts an external or internal wall. The only possible discrete transition is $P'_0 \rightarrow P_1$.

1. Either $\delta_{h'}(v) \neq 0$ and
 - (i) $\eta = \eta'$,
 - (ii) $\pi_v = \frac{1 + \text{sgn}(C_{v,\rho(v,\eta),\eta_v})}{2}$ for all $v \in \text{first}(h')$,
 - (iii) $\forall u \in V \setminus \text{first}(h')$, if $u \notin \text{sv}(h')$ then $\pi_u = \pi'_u + \delta_{h'}(v) \times C_{u,\rho(u,\eta'),\eta'_u}$,
 else $\pi_u = \pi'_u = \frac{1 + \text{sgn}(C_{u,\rho(u,\eta'),\eta'_u})}{2}$.
2. Or $\delta_{h'}(v) = 0$ and
 - (i) $\eta_v = \eta'_v + \text{sgn}(C_{v,\rho(v,\eta'),\eta'_v})$,
 - (ii) $\pi_v = \frac{1 - \text{sgn}(C_{v,\rho(v,\eta'),\eta'_v})}{2}$,
 - (iii) $\forall u \in V \setminus \{v\}$, $\eta_u = \eta'_u$ and $\pi'_u = \pi_u$.

On the one hand, the item 1. of the definition describes the *continuous transitions*: the transitions lead to the last hybrid state inside the current discrete state which could give rise to a qualitative change. On the other hand, the item 2. describes the *discrete transitions*: if the system cannot evolve anymore within the current discrete state, the trajectory goes through a border.

Let us remark that, if v does not encounter any internal or external wall, the time spent in a particular discrete state is computed by the formula $\Delta t = \frac{\pi_v - \pi'_v}{C_{v,\omega,n}}$ where π'_v (resp. π_v) is the fractional part of the variable v at the entrance (resp. exit) of the current state and $C_{v,\omega,n}$ is the celerity of the variable v inside the current discrete state. Δt is called the *duration* of the continuous transition.

3 Hybrid Hoare Logic

3.1 Languages

This section is dedicated to the presentation of the Hoare logic adapted to our hybrid formalism. We first detail the notion of terms (Def. 8) in order to define

the property language (Def. 9) later used for pre- and postconditions, the assertion language (Def. 10) and its semantics (Def. 11) used to describe biological knowledge on a hybrid transition, and the path language (Def. 12) later used to describe an observed trace inside a hybrid gene regulatory network.

Definition 8 (Terms). *The terms are inductively defined as follows:*

- The variables η_u , π_u and π'_u where $u \in V$ are terms;
- The variables $C_{u,\omega,n}$ where $u \in V$, $\omega \subset R^-(u)$ and $n \in \llbracket 0, b_u \rrbracket$ are terms;
- The variables and constants of \mathbb{R} and \mathbb{N} are terms;
- The set of considered connectives that create new terms are: $+$, $-$, \times , $/$.

We denote by \square any of the following symbols: $<$, \leq , $>$, \geq , $=$, \neq . Moreover we note $Terms_d$ (resp. $Terms_h$) the set of “discrete” terms (resp. “hybrid” terms) built on variables η_u and on variables and constants of \mathbb{N} (resp. on variables π_u , π'_u , $C_{u,\omega,n}$ and on variables and constants of \mathbb{R}).

Definition 9 (Property language \mathcal{L}_C). *The atoms of the property language are of two types:*

- Discrete atoms are of the form: $n \square n'$ where $n, n' \in Terms_d$;
- Hybrid atoms are of the form: $f \square f'$ where $f, f' \in Terms_h$;

The discrete conditions are defined by: $D ::= a_d \mid \neg D \mid D \wedge D \mid D \vee D$ where a_d is a discrete atom.

The hybrid conditions are defined by: $H ::= a_d \mid a_h \mid \neg H \mid H \wedge H \mid H \vee H$ where a_d and a_h are respectively a discrete atom and a hybrid one.

A property is a couple (D, H) formed by a discrete and a hybrid condition. All properties form the property language \mathcal{L}_C .

A hybrid state h satisfies a property $\varphi = (D, H) \in \mathcal{L}_C$ iff both D and H hold in h , by using the usual meaning of the connectives; in this case, we note $h \models \varphi$.

Definition 10 (Assertion language \mathcal{L}_A). *The assertion language \mathcal{L}_A is defined by the following grammar:*

$$a ::= \top \mid C_v \square c \mid \text{slide}(v) \mid \text{slide}^+(v) \mid \text{slide}^-(v) \mid \neg a \mid a \wedge a \mid a \vee a$$

where $v \in V$ is a variable name and $c \in \mathbb{R}$ is a real number.

Definition 11 (Semantics of the assertion couple $(\Delta t, a)$). *Let us consider a hybrid state $h' = (\eta, \pi')$ and the unique continuous transition starting from h' and ending in $h = (\eta, \pi)$. The satisfaction relation between the continuous transition $h' \longrightarrow h$ and an assertion couple $(\Delta t, a) \in \mathbb{R}^+ \times \mathcal{L}_A$ is noted $(h', h) \models (\Delta t, a)$, by overloading of notation, and is defined as follows:*

- If $a \equiv \top$, $(h', h) \models (\Delta t, a)$ iff $\delta_{h'}^{first} = \Delta t$.
- If a is of the form $(C_u \square c)$, $(h', h) \models (\Delta t, a)$ iff $\delta_{h'}^{first} = \Delta t$ and $(C_{u,\rho(u,\eta),\eta_u} \square c)$.

- If a is of the form $slide(v)$, $(h', h) \models (\Delta t, a)$ iff $\delta_{h'}^{first} = \Delta t$ and $\delta_{h'}(v) < \delta_{h'}^{first}$.
- If a is of the form $slide^+(v)$ (resp. $slide^-(v)$), $(h', h) \models (\Delta t, a)$ iff $\delta_{h'}^{first} = \Delta t$ and $\delta_{h'}(v) < \delta_{h'}^{first}$ and $C_{v,\rho(v,\eta),\eta_v} > 0$ (resp. $C_{v,\rho(v,\eta),\eta_v} < 0$).
- If a is of the form $\neg a'$, $(h', h) \models (\Delta t, a)$ iff $\delta_{h'}^{first} = \Delta t$ and $(h', h) \not\models (\Delta t, a')$.
- If a is of the form $a' \wedge a''$ (resp. $a' \vee a''$), $(h', h) \models (\Delta t, a)$ iff $(h', h) \models (\Delta t, a')$ and (resp. or) $(h', h) \models (\Delta t, a'')$.

Definition 12 (Path language \mathcal{L}_P). The (discrete) path atoms are defined by:
 $dpa ::= v + \mid v -$ where $v \in V$ is a variable name.

The (discrete) paths are defined by: $p ::= \varepsilon \mid (\Delta t, assert, dpa) \mid p ; p$
 where Δt is either a real constant or a variable, $assert$ is an assertion, and dpa is a discrete path atom.

3.2 Hoare Triples

Using the languages defined in the previous section, we give here the syntax (Def. 13) and the semantics (Def. 14) of a Hoare triple in the scope of our hybrid formalism, which are natural extensions of the classical definitions.

Definition 13 (Hybrid Hoare Triples). A Hoare triple for a given GRN is an expression of the form $\{Pre\} p \{Post\}$ where Pre and $Post$, called precondition and postcondition respectively, are properties of \mathcal{L}_C , and p is a path from \mathcal{L}_P .

Definition 14 (Semantics of Hoare triples). We say that a Hoare triple $\{Pre\} p \{Post\}$ is satisfied if:

- If p is atomic and is of the form $(\Delta t, assert, v+)$ (resp. $(\Delta t, assert, v-)$) then for all $h'_1 = (\eta_1, \pi'_1) \models Pre$, there exists two hybrid states $h_1 = (\eta_1, \pi_1)$ and $h'_2 = (\eta_2, \pi'_2)$ with $\eta_v^2 = \eta_v^1 + 1$ (resp. $\eta_v^2 = \eta_v^1 - 1$) such that:
 - there exists a continuous transition from h'_1 towards h_1 such that $(h'_1, h_1) \models (\Delta t, assert)$,
 - there exists a discrete transition from h_1 towards h'_2 ,
 - $h'_2 \models Post$;
- If $p = p_1; p_2$ is a sequence of paths, then there exist $Post_1$ and Pre_2 of the property language such that both triples $\{Pre\} p_1 \{Post_1\}$ and $\{Pre_2\} p_2 \{Post\}$ are satisfied and $Post_1 \Rightarrow Pre_2$.

Figure 3 represents an execution inside a GRN containing several discrete transitions. It illustrates the fact that both the precondition and postcondition of a particular Hoare triple are associated with the hybrid states corresponding to the entrance in the related discrete states.

3.3 Weakest Precondition Calculus

In this section, we detail the computation of the weakest precondition related to a given program and postcondition. This method is inspired from the original weakest precondition proposed by Dijkstra [8] with several additions allowing

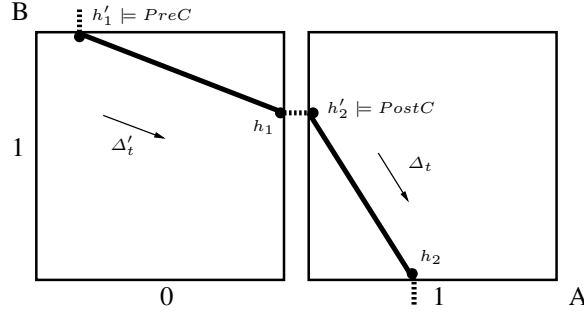


Fig. 3. Hoare triple example: $\{PreC\} (\Delta'_t, \top, A+) \{PostC\}$. Starting from the hybrid state h'_1 , and considering the path in bold line, it is possible to chain a hybrid transition of duration Δ'_t ($h'_1 \rightarrow h_1$) and a discrete transition ($h_1 \rightarrow h'_2$) so that the discrete level of A is increased: this Hoare triple is satisfied.

to take the hybrid dynamics into account. Indeed, given the semantics of the hybrid formalism defined in Sec. 2, several conditions have to be met to allow the execution of a $v+$ or $v-$ instruction: amongst others, the variable v must be the first to be able to cross a border, the celerities must allow the related continuous and discrete transitions, there is a relationship between the fractional parts of states before and after a discrete transition...

In Def. 15, we give a formal definition of the weakest precondition (D', H') of a path program according to a given postcondition (D, H) . The interesting terminal cases are the increment ($v+$) and decrement ($v-$) instructions.

Definition 15 (Weakest precondition). *Let p be a path program and $Post = (D, H_f)$ a property which will have the role of a post-condition parameterized by a final state index f . The weakest precondition attributed to p and $Post$ is a property: $WP_f^i(p, Post) \equiv (D', H'_{i,f})$, parameterized by a fresh initial state index i and the same final state f , and whose value is recursively defined by:*

- If $p = \varepsilon$ is the empty sequence program, then $D' \equiv D$ and $H'_{i,f} \equiv H_f$;
- If $p = (\Delta t, \text{assert}, v+)$ is an atom, with $v \in V$:
 - $D' \equiv D[\eta_v \setminus \eta_v + 1]$,
 - $H'_{i,f} \equiv H_f \wedge \Phi_v^+(\Delta t) \wedge \mathcal{F}_v(\Delta t) \wedge \neg \mathcal{W}_v^+ \wedge \mathcal{A}(\Delta t, \text{assert}) \wedge \mathcal{J}_v$;
- If $p = (\Delta t, \text{assert}, v-)$ is an atom, with $v \in V$:
 - $D' \equiv D[\eta_v \setminus \eta_v - 1]$,
 - $H'_{i,f} \equiv H_f \wedge \Phi_v^-(\Delta t) \wedge \mathcal{F}_v(\Delta t) \wedge \neg \mathcal{W}_v^- \wedge \mathcal{A}(\Delta t, \text{assert}) \wedge \mathcal{J}_v$;
- If $p = p_1; p_2$ is a concatenation of programs:

$$WP_f^i(p_1; p_2, Post) \equiv WP_m^i(p_1, WP_f^m(p_2, Post))$$

which is parameterized by a fresh intermediate state index m ;

where $\Phi_v^+(\Delta t)$, $\Phi_v^-(\Delta t)$, \mathcal{W}_v^+ , \mathcal{W}_v^- , $\mathcal{F}_v(\Delta t)$, $\mathcal{A}(\Delta t, \text{assert})$ and \mathcal{J}_v are sub-properties given in Appendix A.

In the following, we informally describe the content of the sub-properties that are used in the weakest precondition construction. The complete formal definitions are given in Appendix A. It has to be noted that all of these properties implicitly depend on the indices i and f used in Def. 15. In the following, $v \in V$ is a component and $\Delta t \in \mathbb{R}^+$ is a time delay.

- $\Phi_v^+(\Delta t)$ (resp. $\Phi_v^-(\Delta t)$) describes the conditions in which v increases (resp. decreases) its discrete expression level: its celerity in the current state must be positive (resp. negative), and its final fractional part π_v depends on Δt .
- \mathcal{W}_v^+ (resp. \mathcal{W}_v^-) states that there is an internal or external wall preventing v from increasing (resp. decreasing) its qualitative state; this sub-property must of course be false for the discrete transition to take place.
- $\mathcal{F}_v(\Delta t)$ states that v belongs to the set of knocking variables, that is, the variables which can first change their qualitative state; in other words, all components other than v must either reach their border after v , or face an internal or external wall.
- $\mathcal{A}(\Delta t, \text{assert})$ translates all assertion symbols given in *assert* expressing constraints on celerities and slides into property language.
- \mathcal{J}_v establishes a junction between the fractional parts of two successive states linked by a discrete transition: it states that all fractional parts are left the same, except for the variable v performing the transition, whose fractional part swaps between 0 and 1.

3.4 Backward strategy

A Hoare triple $\{Pre\} \text{ path } \{Post\}$ being given, we call backward strategy the proof strategy defined inductively on *path* as follows:

1. If *path* is of the form $(\Delta t, \text{assert}, v+)$, $(\Delta t, \text{assert}, v-)$ or ε , then compute the precondition of *path*;
2. If *path* is of the form $p_1; p_2$ where p_2 is of the form $(\Delta t, \text{assert}, v+)$ or $(\Delta t, \text{assert}, v-)$, then compute the precondition just before p_2 and iterate for path p_1 .

Notice that, these two items being mutually exclusive, the backward strategy generates a unique proof tree. Furthermore, given the semantics of Hoare triples, every path can be seen as completely linear, justifying the backward strategy.

4 Example: Controlling the *lacI* Repressor by *NRIp*

4.1 Presentation

We focus on the *lacI* repressor regulation of the lactose operon in *E. Coli*. An operon is a functional DNA unity which regroups some genes in order to transcribe them one after the other. These genes are regulated by only one promoter allowing a coordinated control of their synthesis. In the case of the lactose operon,

three genes are controlled: *lacZ*, *lacY* and *lacA* which produce the proteins β -galactosidase, permease and thiogalactoside transacetylase, respectively. The β -galactosidase cleaves lactose into glucose and galactose. The permease permits the passive transport of the lactose. Finally, the thiogalactoside transacetylase confers the detoxification of the cell [1].

The lactose operon is controlled by two sites found ahead of the genes: the *lac p* promoter and the *lac o* operator. The RNA polymerase binds to the promoter and activates the operon. On the contrary, the *lacI* repressor binds to the operator and prevents the transcription of the operon genes. In presence of lactose, *lacI* repressing activity is suppressed, allowing the breaking up of the lactose.

Atkinson *et al.* [2] used the lactose operon and the *Ntr* systems to enable oscillatory behaviours (Fig. 1). Vertex *A* encodes the *NRI* protein due to the *glnG* gene combined with the *glnA* promoter. This promoter is regulated by the phosphorylated *NRI* (*NRIp*; self-loop on *A*) and by *lacI* (activation from vertex *B*). Finally, the *lacI* gene repressor, fused with *glnK* promoter (vertex *B*), is regulated by *NRIp*.

Because the *glnK* promoter is activated at high *NRIp* levels [2], we assumed that the auto-activation of *A* is necessary before regulating *B*. In other words, the quantity of *NRIp* should be enough to stimulate *lacI*, which explains the quantitative thresholds indicated into the interaction graph for vertex *A*. Similarly, we only have one threshold crossed for vertex *B*: $\neg(B \geq 1)$, because *lacI* only negatively interacts with the *NRI* gene. A comparative study of the behaviour of β -galactosidase in modelling predictions and in experimental conditions showed same sustained oscillation [12].

These oscillations are known to pass through the following sequence of discrete states: $(A = 2, B = 0)$, $(2, 1)$, $(1, 1)$, $(1, 0)$, $(2, 0)$. Thus, we consider the following Hoare triple (for better readability, tuples are written vertically):

$$\left\{ \begin{array}{c} D_4 \\ H_4 \end{array} \right\} \left(\begin{array}{c} T_4 \\ \top \\ B+ \end{array} \right); \left(\begin{array}{c} T_3 \\ \text{slide}^+(B) \\ A- \end{array} \right); \left(\begin{array}{c} T_2 \\ \top \\ B- \end{array} \right); \left(\begin{array}{c} T_1 \\ \top \\ A+ \end{array} \right) \left\{ \begin{array}{c} D_0 \equiv (\eta_A = 2 \wedge \eta_B = 0) \\ H_0 \equiv \top \end{array} \right\} .$$

$\begin{array}{ccccc} \uparrow & & \uparrow & & \uparrow \\ D_3, H_3 & & D_2, H_2 & & D_1, H_1 \end{array}$

Intuitively, when starting from a state satisfying the discrete part D_4 and the hybrid part H_4 , the system evolves until reaching the state $\eta_A = 2 \wedge \eta_B = 0$ (D_0) and satisfying the hybrid part H_0 . From the initial state, *lacI* is synthesised due to the activation of its gene (transition $B+$), then this repressor inhibits the gene producing the *NRI* protein (transition $A-$). Then, the gene of *lacI* doesn't remain active (transition $B-$) which permits the activation of the gene of the *NRI* protein (transition $A+$), consequently reaching the final state.

We also assumed that the activation of the repressor reached its maximum concentration ($\text{slide}(B)$, see Fig. 4) to act on the lactose operon when *lacI* is present. Finally, the lack of temporal data prevented us to assign a value to the

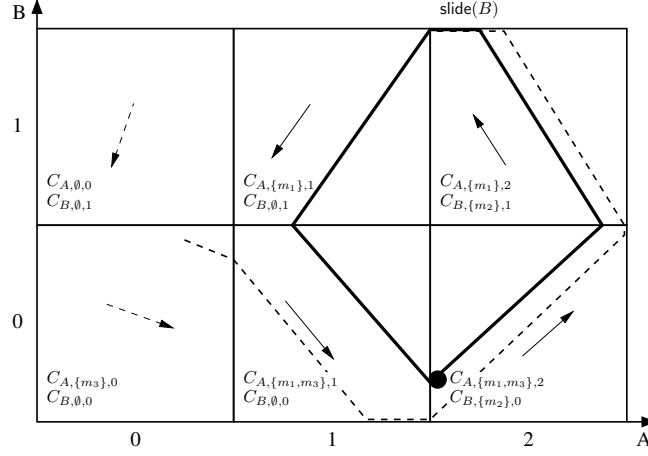


Fig. 4. State graph of the lacI/Ntr system. Here, we suppose that only the sign of celerities is known. The shape of the limit cycle depends on the celerities into each crossed state and on biologically known slides. The black circle is the initial state we used. The dashed lines show that there exist other initial states that do not belong to the limit cycle but whose trajectories end into it.

duration Δt of each phase, therefore we used the constants T_1 , T_2 , T_3 and T_4 for these terms.

Using the backward strategy of Subsec. 3.4 on this Hoare triple allows the determination of each intermediate property (D_i, H_i) . Intuitively, we would like to represent a cyclic behaviour as in Fig. 4, which would lead to make identical the pre- and postconditions. We first compute the weakest precondition (D_4, H_4) of the previous Hoare triple.

4.2 First Step of the Backward Strategy

In this section, we focus on the first step of the backward strategy, corresponding to the following Hoare triple:

$$\left\{ \begin{array}{l} D_1 \\ H_1 \end{array} \right\} \left(\begin{array}{c} T_1 \\ \top \\ A+ \end{array} \right) \left\{ \begin{array}{l} D_0 \equiv (\eta_A = 2 \wedge \eta_B = 0) \\ H_0 \equiv \top \end{array} \right\} .$$

Furthermore, we only show the final result of each sub-formula of the weakest precondition in order to give the reader a hint of the results obtained. Indeed, a lot of simplifications can be made inside these sub-formulas because they depend on the discrete state of each step, which is always fully known in this example, or by using some particularities of our hybrid formalism. The details of the computation for this first step are given in Appendix B.1.

First of all, Def. 15 gives:

$$D_1 \equiv D_0[\eta_A \setminus \eta_A + 1] \equiv (\eta_A + 1 = 2) \wedge (\eta_B = 0) \equiv (\eta_A = 1) \wedge (\eta_B = 0) , \quad (2)$$

$$H_1 \equiv H_0 \wedge \Phi_A^+(T_1) \wedge \mathcal{F}_A(T_1) \wedge \neg \mathcal{W}_A^+ \wedge \mathcal{A}(T_1, \text{assert}) \wedge \mathcal{J}_A . \quad (3)$$

The final value of the interesting sub-formulas are given in the following:

$$\Phi_A^+(T_1) \equiv (\pi_A^1 = 1) \wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = \pi_A^1 - C_{A,\{m_1,m_3\},1} \cdot T_1) , \quad (4)$$

$$\mathcal{F}_A(T_1) \equiv \neg(C_{B,\emptyset,0} > 0) \vee \neg(\pi_B^{1'} > \pi_B^1 - C_{B,\emptyset,0} \cdot T_1) , \quad (5)$$

$$\mathcal{J}_A \equiv (\pi_B^1 = \pi_B^{0'}) \wedge (\pi_A^1 = 1 - \pi_A^{0'}) . \quad (6)$$

Here, (4) gives some requirements for A to increase its discrete level: its final fractional part (π_A^1) is on border 1, its celerity in the current discrete state is positive, and its initial fractional part is directly given by the time T_1 spent in this state. Moreover, (5) states that, because B cannot cross a discrete threshold before A , then it must face a wall, or be too far away from its border to cross it. It is required that A does not meet an internal (or external) wall in this example, which is always the case here because $\neg \mathcal{W}_A^+ \equiv \top$. Furthermore, for this particular path, we have no assertion constraining the continuous transition, thus: $\mathcal{A}(T_1, \text{assert}) \equiv \top$. Finally, (6) links the fractional part of both variables in the current discrete state with their values in the next one. These results lead to the final value of the hybrid part H_1 :

$$\begin{aligned} H_1 \equiv & \left(\neg(C_{B,\emptyset,0} > 0) \vee \neg(\pi_B^{1'} > \pi_B^{0'} - C_{B,\emptyset,0} \cdot T_1) \right) \\ & \wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = 1 - C_{A,\{m_1,m_3\},1} \cdot T_1) \wedge (\pi_A^{0'} = 0) . \end{aligned} \quad (7)$$

Therefore, we obtained constraints for each celerity as well as each fractional part in this current state.

4.3 Final Result

Using the backward strategy, we calculated the constraints of the discrete part D_4 and the hybrid part H_4 step by step (all details are given in Appendix B.2, B.3 and B.4). We also took into account the knowledge of the limit cycle in order to add another particular constraint (see Appendix B.5). The final result is the

following:

$$\begin{aligned}
H_F \equiv & (\neg(C_{B,\emptyset,0} > 0) \vee \neg(1 > \pi_B^{0'} - C_{B,\emptyset,0} \cdot T_1)) \\
& \wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = 1 - C_{A,\{m_1,m_3\},1} \cdot T_1) \\
& \wedge (\neg(C_{A,\{m_1\},1} > 0) \vee \neg(1 > \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2)) \\
& \wedge ((C_{A,\emptyset,0} > 0) \vee \neg(C_{A,\{m_1\},1} < 0) \vee \neg(1 < \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2)) \\
& \wedge (C_{B,\emptyset,1} < 0) \wedge (1 = 0 - C_{B,\emptyset,1} \cdot T_2) \\
& \wedge (\neg(C_{B,\{m_2\},1} < 0) \vee \neg(0 < 1 - C_{B,\{m_2\},1} \cdot T_3)) \\
& \wedge (C_{A,\{m_1\},2} < 0 \wedge (\pi_A^{3'} = 0 - C_{A,\{m_1\},2} \cdot T_3)) \\
& \wedge (\neg(C_{B,\{m_2\},1} > 0) \vee (0 > 1 - C_{B,\{m_2\},1} \cdot T_3)) \\
& \wedge (\neg(C_{A,\{m_1,m_3\},2} < 0) \vee \neg(0 < \pi_A^{3'} - C_{A,\{m_1,m_3\},2} \cdot T_4)) \\
& \wedge (C_{B,\{m_2\},0} > 0) \wedge (\pi_B^{0'} = 1 - C_{B,\{m_2\},0} \cdot T_4) .
\end{aligned} \tag{8}$$

Each celerity included in this biological system is mentioned in a constraint depending on the temporal information and on the fractional parts of the corresponding variable inside the related discrete state. The constraints are established for the limit cycle. A constraint solver will then be useful to reduce the formulas and identify the sets of exact values which can be attributed to the celerities. Once a suitable set of parameters is chosen, a comparison between the simulation of this model and biological experiments should be carried out to assess if the behaviours are similar.

5 Conclusion

In this paper, we developed a suitable approach based on biological data taking into account durations to identify new constraints on parameters piloting the dynamics of the model. We proved the usefulness of our hybrid Hoare logic by determining the constraints on celerities of a small interaction graph between the *lacI* repressor and the *Ntr* system. Indeed, our backward strategy leads to a constraint for every celerity encountered along the observed cyclic behaviour.

To strengthen our formalism, we now have to prove the soundness and correctness of our weakest precondition calculus. One of the drawbacks of our Hoare logic resides in the size of the obtained precondition. It becomes mandatory to simplify on the fly all intermediate formulas. To go further, it would be useful to give the obtained weakest precondition to a constraint solver in order to generate one or several solutions. Thereafter one can automate the identification of celerities and simulations to compare the simulated traces with experimental biological data. This global process has already been performed manually on a model of the cell cycle in mammals [3].

Beyond the sequential path studied into this paper, our approach may be extended to the conditional branching instruction (*if* [] *then* [] *else* []), or the iteration instruction (*while* [] *do* []) as it was already made in the discrete

case [5]. This should lead to an expressive framework in which durations between two experimental measures, as well as complex behaviours, are well taken into consideration.

Acknowledgements. We are grateful to E. Cornillon and G. Bernot for fruitful discussions about the hybrid formalism. This work is partially supported by the French National Agency for Research (ANR-14-CE09-0011).

References

1. K.J. Andrews and E.C. Lin. Thiogalactoside transacetylase of the lactose operon as an enzyme for detoxification. *J. bacteriol.*, 128(1):510, 1976.
2. Mariette R. Atkinson, Michael A. Savageau, Jesse T. Myers, and Alexander J. Ninfa. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *escherichia coli*. *Cell*, 113(5):597–607, 2003.
3. J. Behaegel, J.-P. Comet, G. Bernot, E. Cornillon, and F. Delaunay. A hybrid model of cell cycle in mammals. *J. Bioinformatics and Comput. Biol.*, 14(1):1640001 [17 pp.], 2016.
4. G. Bernot, J.-P. Comet, Z. Khalis, A. Richard, and O. Roux. A genetically modified Hoare logic. *ArXiv: 1506.05887*, June 2015.
5. G. Bernot, J.-P. Comet, and O. Roux. A genetically modified Hoare logic that identifies the parameters of a gene networks. In O. Roux and J. Bourdon, editors, *CMSB’15*, volume 9308 of *LNBI*, pages 8–12, 2015.
6. J.-P. Comet, G. Bernot, A. Das, F. Diener, C. Massot, and A. Cessieux. Simplified models for the mammalian circadian clock. In *Proceedings of CSBio’2012*, volume 11 of *Procedia Computer Science*, pages 127–138, Bangkok (Thailand), October 3–5 2012.
7. E. Cornillon, J.-P. Comet, G. Bernot, and G. Enée. *Proc. of the Thematic Research School on Advances in Systems and Synthetic Biology, Modelling Complex Biological Systems in the Context of Genomics*, chapter Hybrid Gene Networks: a new Framework and a Software Environment, pages 57–84. EDP Sciences, ISBN : 978-2-7598-1971-3, 2016.
8. Edsger W. Dijkstra. Guarded commands, nondeterminacy and formal derivation of programs. *Commun. ACM*, 18:453–457, August 1975.
9. C.A.R. Hoare. An axiomatic basis for computer programming. *Communications of the ACM*, 12(10):576–585, oct 1969.
10. Daisuke Ishii, Guillaume Melquiond, and Shin Nakajima. Inductive verification of hybrid automata with strongest postcondition calculus. In *Proceedings of IFM 2013*, pages 139–153. Springer Berlin Heidelberg, 2013.
11. U. Martin, E.A. Mathiesen, and P. Oliva. Hoare logic in the abstract. In *EACSL’06*, pages 501–515. Springer Berlin Heidelberg, 2006.
12. Oliver Purcell, Nigel J. Savery, Claire S. Grierson, and Mario di Bernardo. A comparative analysis of synthetic genetic oscillators. *Journal of The Royal Society Interface*, 7(52):1503–1524, 2010.
13. R. Thomas and M. Kaufman. Multistationarity, the basis of cell differentiation and memory. II. logical analysis of regulatory networks in terms of feedback circuits. *Chaos*, 11:180–195, 2001.
14. Liang Zou, Naijun Zhan, Shuling Wang, Martin Fränzle, and Shengchao Qin. Verifying simulink diagrams via a hybrid hoare logic prover. In *Proceedings of EMSOFT’13*, Piscataway, NJ, USA, 2013. IEEE Press.

Appendix

A Sub-properties of the Weakest Precondition Calculus

In this appendix, we detail the formal content of the sub-properties $\Phi_v^+(\Delta t)$, $\Phi_v^-(\Delta t)$, $\mathcal{F}_v(\Delta t)$, \mathcal{W}_v^+ , \mathcal{W}_v^- , $\mathcal{A}(\Delta t, \text{assert})$ and \mathcal{J}_v informally presented in Subsec. 3.3.

It has to be noted that all of these properties depend on the indices i and f used in Def. 15, although for readability issues we did not mention them on the names of each sub-property. Furthermore, for a given index i , we call by convention π_u^i (resp. $\pi_u^{i'}$) the fractional part of the exiting (resp. entering) state inside the discrete state i . Finally, we recall that Φ_u^ω is true iff the resources of u in the current state are exactly ω , as formally expressed in Def. 3.

A.1 Discrete Transition to the Next Discrete State

For all component $v \in V$, $\Phi_v^+(\Delta t)$ (resp. $\Phi_v^-(\Delta t)$) describes the conditions in which v increases (resp. decreases) its discrete expression level: its celerity in the current state must be positive (resp. negative) and its fractional part only depends on Δt in the way given at the very end of Sec. 2.

$$\begin{aligned} \Phi_v^+(\Delta t) &\equiv (\pi_v^i = 1) \\ &\quad \wedge \bigwedge_{\substack{\omega \in R^-(v) \\ n \in \llbracket 0, b_v \rrbracket}} \left((\Phi_v^\omega \wedge (\eta_v = n)) \Rightarrow (C_{v,\omega,n} > 0) \wedge (\pi_v^{i'} = \pi_v^i - C_{v,\omega,n} \cdot \Delta t) \right) , \\ \Phi_v^-(\Delta t) &\equiv (\pi_v^i = 0) \\ &\quad \wedge \bigwedge_{\substack{\omega \in R^-(v) \\ n \in \llbracket 0, b_v \rrbracket}} \left((\Phi_v^\omega \wedge (\eta_v = n)) \Rightarrow (C_{v,\omega,n} < 0) \wedge (\pi_v^{i'} = \pi_v^i - C_{v,\omega,n} \cdot \Delta t) \right) . \end{aligned}$$

A.2 Internal and External Walls

For all component $u \in V$, \mathcal{W}_u^+ (resp. \mathcal{W}_u^-) states that there is a wall preventing u to increase (resp. decrease) its qualitative state. This wall can either be an external wall EW_u^+ (resp. EW_u^-) or an internal wall IW_u^+ (resp. IW_u^-). Furthermore, $\Phi_{u+}^{\omega'}$ (resp. $\Phi_{u-}^{\omega'}$), which is required in these sub-formulas, is true if and only if the set of resources of u is exactly ω' in the state where u is increased (resp. decreased) by 1.

$$\mathcal{W}_u^+ \equiv \text{IW}_u^+ \vee \text{EW}_u^+ \quad \text{and} \quad \mathcal{W}_u^- \equiv \text{IW}_u^- \vee \text{EW}_u^-$$

where:

$$\begin{aligned} \text{EW}_u^+ &\equiv (\eta_u = b_u) \wedge \bigwedge_{\omega \in R^-(u)} (\Phi_u^\omega \Rightarrow C_{u,\omega,b_u} > 0) , \\ \text{EW}_u^- &\equiv (\eta_u = 0) \wedge \bigwedge_{\omega \in R^-(u)} (\Phi_u^\omega \Rightarrow C_{u,\omega,0} < 0) , \end{aligned}$$

$$\begin{aligned}
IW_u^+ &\equiv (\eta_u < b_u) \wedge \bigwedge_{\substack{\omega, \omega' \in R^-(u) \\ n \in \llbracket 0, b_u \rrbracket}} \left(((\eta_u = n) \wedge (m = n + 1) \wedge \Phi_u^\omega \wedge \Phi_{u+}^{\omega'}) \right. \\
&\quad \left. \Rightarrow C_{u, \omega, n} > 0 \wedge C_{u, \omega', m} < 0 \right) , \\
IW_u^- &\equiv (\eta_u > 0) \wedge \bigwedge_{\substack{\omega, \omega' \in R^-(u) \\ n \in \llbracket 0, b_u \rrbracket}} \left(((\eta_u = n) \wedge (m = n - 1) \wedge \Phi_u^\omega \wedge \Phi_{u-}^{\omega'}) \right. \\
&\quad \left. \Rightarrow C_{u, \omega, n} < 0 \wedge C_{u, \omega', m} > 0 \right) ,
\end{aligned}$$

$$\begin{aligned}
\Phi_{u+}^{\omega'} &\equiv (\eta_u < b_u) \wedge \bigwedge_{n \in \llbracket 0, b_u \rrbracket} ((\eta_u = n) \Rightarrow \Phi_u^{\omega'}[\eta_u \setminus \eta_u + 1]) , \\
\Phi_{u-}^{\omega'} &\equiv (\eta_u > 0) \wedge \bigwedge_{n \in \llbracket 0, b_u \rrbracket} ((\eta_u = n) \Rightarrow \Phi_u^{\omega'}[\eta_u \setminus \eta_u - 1]) .
\end{aligned}$$

A.3 Knocking Variable Able to Perform a Discrete Transition

For all component $v \in V$, $\mathcal{F}_v(\Delta t)$ states that all components other than v must either reach their border after v , or face an internal or external wall. In other words, v belongs to the set of components which can first change its qualitative state. This is required to enforce the meaning of an instruction $v+$ or $v-$, which means that no other component changes its qualitative state before v .

$$\begin{aligned}
\mathcal{F}_v(\Delta t) &\equiv \bigwedge_{u \in V \setminus \{v\}} \left(\bigwedge_{\substack{\omega \in R^-(u) \\ n \in \llbracket 0, b_u \rrbracket}} ((\eta_u = n) \wedge \Phi_u^\omega \wedge C_{u, \omega, n} > 0 \wedge \pi'_{u, i} > \pi_{u, i} - C_{u, \omega, n} \cdot \Delta t) \Rightarrow \mathcal{W}_u^+ \right) \\
&\quad \wedge \left(\bigwedge_{\substack{\omega \in R^-(u) \\ n \in \llbracket 0, b_u \rrbracket}} ((\eta_u = n) \wedge \Phi_u^\omega \wedge C_{u, \omega, n} < 0 \wedge \pi'_{u, i} < \pi_{u, i} - C_{u, \omega, n} \cdot \Delta t) \Rightarrow \mathcal{W}_u^- \right) .
\end{aligned}$$

A.4 Hybrid Assertions

The sub-property $\mathcal{A}(\Delta t, a)$ allows one to translate all assertion symbols given about the continuous transition related to the instruction (celerities and slides) into a property:

$$\mathcal{A}(\Delta t, a) \equiv \bigwedge_{\substack{u \in V \\ n_u \in \llbracket 0, b_u \rrbracket \\ \omega_u \in R^-(u)}} \left(\bigwedge_{\substack{v \in V \\ n_v \in \llbracket 0, b_v \rrbracket \\ \omega_v \in R^-(v)}} ((\eta_v = n_v) \wedge \Phi_v^{\omega_v} \Rightarrow a[C_v \setminus C_{v, \omega_v, n_v}]) \right) \begin{bmatrix} \text{slide}(u) \setminus \mathcal{S}_{u, \omega_u, n_u} \\ \text{slide}^+(u) \setminus \mathcal{S}_{u, \omega_u, n_u}^+ \\ \text{slide}^-(u) \setminus \mathcal{S}_{u, \omega_u, n_u}^- \end{bmatrix}$$

where A is the assert part of the instruction, that is, $P = (\Delta t, A, v+)$ or $P = (\Delta t, A, v-)$, and, for all variable $u \in V$:

$$\begin{aligned}\mathcal{S}_{u,\omega_u,n_u}^+(\Delta t) &\equiv \pi_u^i = 1 \wedge (C_{u,\omega,n} > 0 \Rightarrow \pi_u^{i'} > \pi_u^i - C_{u,\omega,n} \cdot \Delta t) , \\ \mathcal{S}_{u,\omega_u,n_u}^-(\Delta t) &\equiv \pi_u^i = 0 \wedge (C_{u,\omega,n} < 0 \Rightarrow \pi_u^{i'} < \pi_u^i - C_{u,\omega,n} \cdot \Delta t) , \\ \mathcal{S}_{u,\omega_u,n_u}(\Delta t) &\equiv \mathcal{S}_{u,\omega_u,n_u}^+(\Delta t) \vee \mathcal{S}_{u,\omega_u,n_u}^-(\Delta t) .\end{aligned}$$

A.5 Junctions Between Discrete States

For all component $v \in V$, and for a discrete transition happening on component v between an initial and a final state corresponding to the indices i and f , \mathcal{J}_v establishes a junction between the fractional parts of these states. It states that the fractional part of v switches from 1 to 0 for an increase, or from 0 to 1 for a decrease, and all other fractional parts are unchanged:

$$\mathcal{J}_v \equiv (\pi_v^f = 1 - \pi_v^{i'}) \wedge \bigwedge_{u \in V \setminus \{v\}} (\pi_u^f = \pi_u^{i'}) .$$

The phenomenon described by \mathcal{J}_v can be easily observed on Fig. 3 on the discrete transition in the centre: all fractional parts are left the same, except for the variable performing the transition.

B Computation Steps of the *lacI* Repressor Example

B.1 Computation of D_1 and H_1

We detail here the computations that lead to the results summed up in Subsec. 4.2. For better readability, we simplified the numerous conjunctions produced by removing the terms that were trivially true, mainly due to implications with a false premise because of terms such as Φ_v^ω , Φ_{v+}^ω , Φ_{v-}^ω , $m = int$, $\eta_{var} = int$ and $C_{v,\omega,n} \sqsubseteq cste$. In addition, the notation $[...]$ depicts a sub-property which is not expanded because it is in conjunction with another trivially false property. This step concerns the first step of the Hoare triple previously presented, which corresponds to:

$$\left\{ \begin{array}{l} D_1 \\ H_1 \end{array} \right\} \left(\begin{array}{c} T_1 \\ \top \\ A+ \end{array} \right) \left\{ \begin{array}{l} D_0 \equiv (\eta_A = 2 \wedge \eta_B = 0) \\ H_0 \equiv \top \end{array} \right\} .$$

At this point, Def. 15 permits to find the weakest precondition, which is given by:

$$D_1 \equiv D_0[\eta_A \setminus \eta_A + 1] \equiv (\eta_A + 1 = 2) \wedge (\eta_B = 0) \equiv (\eta_A = 1) \wedge (\eta_B = 0) , \quad (9)$$

$$H_1 \equiv H_0 \wedge \Phi_A^+(T_1) \wedge \mathcal{F}_A(T_1) \wedge \neg \mathcal{W}_A^+ \wedge \mathcal{A}(T_1) \wedge \mathcal{J}_A . \quad (10)$$

Firstly, we calculated each element of the hybrid part H_1 . The EW and IW formulas (formally given in Subsec. A.2) are computed for both variables. These formulas will be used thereafter in the sub-formulas $\mathcal{F}_A(T_1)$ and $\neg\mathcal{W}_A^+$.

$$\begin{aligned}
IW_A^+ &\equiv \left((\eta_A < b_A) \wedge ((\eta_A = 1) \wedge (m = 2) \wedge \Phi_A^{m_1, m_3} \wedge \Phi_{A+}^{m_1, m_3}) \right. \\
&\quad \left. \Rightarrow C_{A, \{m_1, m_3\}, 1} > 0 \wedge C_{A, \{m_1, m_3\}, 2} < 0 \right) \equiv \perp \\
IW_B^- &\equiv (\eta_B > 0) \wedge [\dots] \equiv \perp \\
IW_B^+ &\equiv \left((\eta_B < b_B) \wedge ((\eta_B = 0) \wedge (m = 1) \wedge \Phi_B^\emptyset \wedge \Phi_{B+}^\emptyset) \right. \\
&\quad \left. \Rightarrow C_{B, \emptyset, 0} > 0 \wedge C_{B, \emptyset, 1} < 0 \right) \equiv \perp \\
EW_A^+ &\equiv (\eta_A = b_A) \wedge [\dots] \equiv \perp \\
EW_B^- &\equiv (\eta_B = 0) \wedge (\Phi_B^\emptyset \Rightarrow C_{B, \emptyset, 0} < 0) \equiv C_{B, \emptyset, 0} < 0 \\
EW_B^+ &\equiv (\eta_B = b_B) \wedge [\dots] \equiv \perp
\end{aligned}$$

Formulas IW_A^+ and IW_B^+ are false because all the conditions in the premises are true but their conclusion are false. Indeed, celerities having the same resources despite a different qualitative level should have the same nonzero sign (see Def. 1). Thus, $(C_{A, \{m_1, m_3\}, 1} > 0 \wedge C_{A, \{m_1, m_3\}, 2} < 0)$ as well as $(C_{B, \emptyset, 0} > 0 \wedge C_{B, \emptyset, 1} < 0)$ can be reduced to false.

Moreover, formulas $(\eta_A = b_A)$, $(\eta_B = b_B)$ and $(\eta_B > 0)$ are false because $(\eta_A = 1 < b_A)$ and $(\eta_B = 0)$ in the considered state which is $(\eta_A = 1) \wedge (\eta_B = 0)$, therefore EW_A^+ , EW_B^+ and IW_B^- are false. Lastly, the computation of EW_B^- gives the constraint $C_{B, \emptyset, 0} < 0$.

Using the formulas of Subsec. A.2 and the previous results, we deduce that $\neg\mathcal{W}_A^+ \equiv \neg IW_A^+ \wedge \neg EW_A^+ \equiv \top$, $\mathcal{W}_B^+ \equiv \perp$ and $\mathcal{W}_B^- \equiv C_{B, \emptyset, 0} < 0$.

– Computation of $\mathcal{F}_A(T_1)$: Because $(\eta_B = 0)$ and Φ_B^\emptyset are *true*, we have

$$\begin{aligned}
\mathcal{F}_A(T_1) &\equiv ((C_{B, \emptyset, 0} > 0) \wedge (\pi_B^{1'} > \pi_B^1 - C_{B, \emptyset, 0} \cdot T_1)) \Rightarrow \mathcal{W}_B^+ \\
&\quad \wedge ((C_{B, \emptyset, 0} < 0) \wedge (\pi_B^{1'} < \pi_B^1 - C_{B, \emptyset, 0} \cdot T_1)) \Rightarrow \mathcal{W}_B^- \quad (11) \\
&\equiv \neg(C_{B, \emptyset, 0} > 0) \vee \neg(\pi_B^{1'} > \pi_B^1 - C_{B, \emptyset, 0} \cdot T_1) .
\end{aligned}$$

– Computation of $\Phi_A^+(T_1)$:

$$\begin{aligned}
\Phi_A^+(T_1) &\equiv (\pi_A^1 = 1) \wedge \left(\Phi_A^{\{m_1, m_3\}} \wedge (\eta_A = 1) \right. \\
&\quad \left. \Rightarrow (C_{A, \{m_1, m_3\}, 1} > 0) \wedge (\pi_A^{1'} = \pi_A^1 - C_{A, \{m_1, m_3\}, 1} \cdot T_1) \right) . \quad (12)
\end{aligned}$$

Because $\Phi_A^{\{m_1, m_3\}} \equiv \top$ and $(\eta_A = 1) \equiv \top$, we deduce:

$$\Phi_A^+(T_1) \equiv (\pi_A^1 = 1) \wedge (C_{A, \{m_1, m_3\}, 1} > 0) \wedge (\pi_A^{1'} = \pi_A^1 - C_{A, \{m_1, m_3\}, 1} \cdot T_1) .$$

– Computation of \mathcal{J}_A :

$$\mathcal{J}_A \equiv (\pi_B^1 = \pi_B^{0'}) \wedge (\pi_A^1 = 1 - \pi_A^{0'}) . \quad (13)$$

Equation (13) links the fractional part of B in the current discrete state with the next one of the execution path. The fractional part of A is also linked to the next state, whose value is known due to the formula of Subsec. A.1.

- For this particular path, $\mathcal{A}(T_1) \equiv \top$.

We can deduce the value of the hybrid part H_1 :

$$H_1 \equiv \left(\neg(C_{B,\emptyset,0} > 0) \vee \neg(\pi_B^{1'} > \pi_B^{0'} - C_{B,\emptyset,0} \cdot T_1) \right) \quad (14)$$

$$\wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = 1 - C_{A,\{m_1,m_3\},1} \cdot T_1) \wedge (\pi_A^{0'} = 0) .$$

Therefore, we obtained constraints for each celerity as well as each fractional part of this current state.

B.2 Computation of D_2 and H_2

$$\left\{ \begin{array}{c} D_2 \\ H_2 \end{array} \right\} \left(\begin{array}{c} T_2 \\ \top \\ B- \end{array} \right) \left\{ \begin{array}{c} D_1 \equiv (\eta_A = 1 \wedge \eta_B = 0) \\ H_1 \end{array} \right\}$$

$$D_2 \equiv D_1[\eta_B \setminus \eta_B - 1] \equiv (\eta_A = 1) \wedge (\eta_B - 1 = 0) \equiv (\eta_A = 1) \wedge (\eta_B = 1) \quad (15)$$

$$H_2 \equiv H_1 \wedge \mathcal{F}_B(T_2) \wedge \Phi_B^-(T_2) \wedge \neg \mathcal{W}_B^- \wedge \mathcal{A}(T_2) \wedge \mathcal{J}_B \quad (16)$$

We calculated the formulas necessary to define $\mathcal{F}_B(T_2)$ and $\neg \mathcal{W}_B^-$:

$$\begin{aligned} IW_A^- &\equiv (\eta_A > 0) \wedge ((\eta_A = 1) \wedge (m = 0) \wedge \Phi_A^{m_1} \wedge \Phi_{A-}^\emptyset \\ &\quad \Rightarrow C_{A,\{m_1\},1} < 0 \wedge C_{A,\emptyset,0} > 0) \\ IW_A^+ &\equiv (\eta_A < b_A) \wedge ((\eta_A = 1) \wedge (m = 2) \wedge \Phi_A^{m_1} \wedge \Phi_{A+}^{m_1} \\ &\quad \Rightarrow C_{A,\{m_1\},1} > 0 \wedge C_{A,\{m_1\},2} < 0) \\ &\equiv \perp \\ IW_B^- &\equiv (\eta_B > 0) \wedge ((\eta_B = 1) \wedge (m = 0) \wedge \Phi_B^\emptyset \wedge \Phi_{B-}^\emptyset \\ &\quad \Rightarrow C_{B,\emptyset,1} < 0 \wedge C_{B,\emptyset,0} > 0) \\ &\equiv \perp \\ EW_A^- &\equiv (\eta_A = 0) \wedge [\dots] \equiv \perp \\ EW_A^+ &\equiv (\eta_A = b_A) \wedge [\dots] \equiv \perp \\ EW_B^- &\equiv (\eta_B = 0) \wedge [\dots] \equiv \perp \end{aligned}$$

With these results, we deduce that $\neg \mathcal{W}_B^- \equiv \neg IW_B^- \wedge \neg EW_B^- \equiv \top$.

– Computation of $\mathcal{F}_B(T_2)$:

$$\begin{aligned}\mathcal{F}_B(T_2) &\equiv ((\eta_A = 1) \wedge \Phi_A^{m_1} \wedge (C_{A,\{m_1\},1} > 0) \wedge (\pi_A^{2'} > \pi_A^2 - C_{A,\{m_1\},1} \cdot T_2)) \\ &\quad \Rightarrow \mathcal{W}_A^+ \\ &\quad \wedge ((\eta_A = 1) \wedge \Phi_A^{m_1} \wedge (C_{A,\{m_1\},1} < 0) \wedge (\pi_A^{2'} < \pi_A^2 - C_{A,\{m_1\},1} \cdot T_2)) \\ &\quad \Rightarrow \mathcal{W}_A^- \\ &\tag{17}\end{aligned}$$

We deduce that $\mathcal{F}_B(T_2) \equiv (\neg(C_{A,\{m_1\},1} > 0) \vee \neg(\pi_A^{2'} > \pi_A^2 - C_{A,\{m_1\},1} \cdot T_2)) \wedge ((C_{A,\emptyset,0} > 0) \vee \neg(C_{A,\{m_1\},1} < 0) \vee \neg(\pi_A^{2'} < \pi_A^2 - C_{A,\{m_1\},1} \cdot T_2))$.

– Computation of $\Phi_B^-(T_2)$:

$$\begin{aligned}\Phi_B^-(T_2) &\equiv (\pi_B^2 = 0) \wedge (\Phi_B^\emptyset \wedge (\eta_B = 1) \\ &\quad \Rightarrow (C_{B,\emptyset,1} < 0) \wedge (\pi_B^{2'} = \pi_B^2 - C_{B,\emptyset,1} \cdot T_2)) \\ &\tag{18}\end{aligned}$$

Because $\Phi_B^\emptyset \equiv \top$ and $(\eta_B = 1) \equiv \top$, we deduce:

$$\Phi_B^-(T_2) \equiv (\pi_B^2 = 0) \wedge (C_{B,\emptyset,1} < 0) \wedge (\pi_B^{2'} = \pi_B^2 - C_{B,\emptyset,1} \cdot T_2)$$

– Computation of \mathcal{J}_B :

$$\mathcal{J}_B \equiv (\pi_A^2 = \pi_A^{1'}) \wedge (\pi_B^2 = 1 - \pi_B^{1'}) \tag{19}$$

– For this particular path, $\mathcal{A}(T_2) \equiv \top$.

We can deduce the value of the hybrid part H_2 :

$$\begin{aligned}H_2 &\equiv H_1 \wedge (\neg(C_{A,\{m_1\},1} > 0) \vee \neg(\pi_A^{2'} > \pi_A^2 - C_{A,\{m_1\},1} \cdot T_2)) \\ &\quad \wedge ((C_{A,\emptyset,0} > 0) \vee \neg(C_{A,\{m_1\},1} < 0) \vee \neg(\pi_A^{2'} < \pi_A^2 - C_{A,\{m_1\},1} \cdot T_2)) \\ &\quad \wedge (\pi_B^2 = 0) \wedge (C_{B,\emptyset,1} < 0) \wedge (\pi_B^{2'} = \pi_B^2 - C_{B,\emptyset,1} \cdot T_2) \\ &\quad \wedge (\pi_A^2 = \pi_A^{1'}) \wedge (\pi_B^{1'} = 1) \\ &\tag{20}\end{aligned}$$

$$\begin{aligned}H_2 &\equiv (\neg(C_{B,\emptyset,0} > 0) \vee \neg(1 > \pi_B^{0'} - C_{B,\emptyset,0} \cdot T_1)) \\ &\quad \wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = 1 - C_{A,\{m_1,m_3\},1} \cdot T_1) \wedge (\pi_A^{0'} = 0) \\ &\quad \wedge (\neg(C_{A,\{m_1\},1} > 0) \vee \neg(\pi_A^{2'} > \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2)) \\ &\quad \wedge ((C_{A,\emptyset,0} > 0) \vee \neg(C_{A,\{m_1\},1} < 0) \vee \neg(\pi_A^{2'} < \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2)) \\ &\quad \wedge (C_{B,\emptyset,1} < 0) \wedge (\pi_B^{2'} = 0 - C_{B,\emptyset,1} \cdot T_2) \\ &\tag{21}\end{aligned}$$

B.3 Computation of D_3 and H_3

$$\left\{ \begin{matrix} D_3 \\ H_3 \end{matrix} \right\} \left(\begin{matrix} T_3 \\ \text{slide}^+(B) \\ A- \end{matrix} \right) \left\{ \begin{matrix} D_2 \equiv (\eta_A = 1 \wedge \eta_B = 1) \\ H_2 \end{matrix} \right\}$$

$$D_3 \equiv D_2[\eta_A \setminus \eta_A - 1] \equiv (\eta_A - 1 = 1) \wedge (\eta_B = 1) \equiv (\eta_A = 2) \wedge (\eta_B = 1) \quad (22)$$

$$H_3 \equiv H_2 \wedge \mathcal{F}_A(T_3) \wedge \Phi_A^-(T_3) \wedge \neg \mathcal{W}_A^- \wedge \mathcal{A}(T_3) \wedge \mathcal{J}_A \quad (23)$$

We calculated the formulas necessary to define $\mathcal{F}_A(T_3)$ and $\neg \mathcal{W}_A^-$:

$$\begin{aligned} \text{IW}_A^- &\equiv (\eta_A > 0) \wedge ((\eta_A = 2) \wedge (m = 1) \wedge \Phi_A^{m_1} \wedge \Phi_{A-}^{m_1} \\ &\quad \Rightarrow C_{A,\{m_1\},2} < 0 \wedge C_{A,\{m_1\},1} > 0) \\ &\equiv \perp \\ \text{IW}_B^- &\equiv (\eta_B > 0) \wedge (\eta_B = 1 \wedge m = 0 \wedge \Phi_B^{m_2} \wedge \Phi_{B-}^{m_2} \\ &\quad \Rightarrow C_{B,\{m_2\},1} < 0 \wedge C_{B,\{m_2\},0} > 0) \\ &\equiv \perp \\ \text{IW}_B^+ &\equiv (\eta_B < b_B) \wedge [\dots] \equiv \perp \\ \text{EW}_A^- &\equiv (\eta_A = 0) \wedge [\dots] \equiv \perp \\ \text{EW}_B^- &\equiv (\eta_B = 0) \wedge [\dots] \equiv \perp \\ \text{EW}_B^+ &\equiv (\eta_B = b_B) \wedge (\Phi_B^{m_2} \Rightarrow C_{B,\{m_2\},1} > 0) \end{aligned}$$

With these results, we deduce that $\neg \mathcal{W}_A^- \equiv \neg \text{IW}_A^- \wedge \neg \text{EW}_A^- \equiv \top$.

– Computation of $\mathcal{F}_A(T_3)$:

$$\begin{aligned} \mathcal{F}_A(T_3) &\equiv ((\eta_B = 1) \wedge \Phi_B^{m_2} \wedge (C_{B,\{m_2\},1} > 0) \wedge (\pi_B^{3'} > \pi_B^3 - C_{B,\{m_2\},1} \cdot T_3)) \\ &\quad \Rightarrow \mathcal{W}_B^+ \\ &\quad \wedge ((\eta_B = 1) \wedge \Phi_B^{m_2} \wedge (C_{B,\{m_2\},1} < 0) \wedge (\pi_B^{3'} < \pi_B^3 - C_{B,\{m_2\},1} \cdot T_3)) \\ &\quad \Rightarrow \mathcal{W}_B^- \end{aligned} \quad (24)$$

We deduce that $\mathcal{F}_A(T_3) \equiv (\neg(C_{B,\{m_2\},1} < 0) \vee \neg(\pi_B^{3'} < \pi_B^3 - C_{B,\{m_2\},1} \cdot T_3))$.

– Computation of $\Phi_A^-(T_3)$:

$$\begin{aligned} \Phi_A^-(T_3) &\equiv (\pi_A^3 = 0) \wedge (\Phi_A^{m_1} \wedge (\eta_A = 2)) \\ &\quad \Rightarrow (C_{A,\{m_1\},2} < 0) \wedge (\pi_A^{3'} = \pi_A^3 - C_{A,\{m_1\},2} \cdot T_3) \end{aligned} \quad (25)$$

Because $\Phi_A^{m_1} \equiv \top$ and $(\eta_A = 2) \equiv \top$, we deduce:

$$\Phi_A^-(T_3) \equiv (\pi_A^3 = 0) \wedge (C_{A,\{m_1\},2} < 0 \wedge (\pi_A^{3'} = \pi_A^3 - C_{A,\{m_1\},2} \cdot T_3))$$

– Computation of \mathcal{J}_A :

$$\mathcal{J}_A \equiv (\pi_B^3 = \pi_B^{2'}) \wedge (\pi_A^3 = 1 - \pi_A^{2'}) \quad (26)$$

– Computation of $\mathcal{A}(T_3)$:

$$\begin{aligned}
\mathcal{A}(T_3) &\equiv \mathcal{S}_B^+ \\
&\equiv (\pi_B^3 = 1) \wedge ((\eta_B = 1) \wedge \Phi_B^{m_2} \\
&\quad \Rightarrow (C_{B,\{m_2\},1} > 0 \Rightarrow \pi_B^{3'} > \pi_B^3 - C_{B,\{m_2\},1} \cdot T_3)) \\
&\equiv (\pi_B^3 = 1) \\
&\quad \wedge \left(\neg(C_{B,\{m_2\},1} > 0) \vee (\pi_B^{3'} > \pi_B^3 - C_{B,\{m_2\},1} \cdot T_3) \right)
\end{aligned} \tag{27}$$

We can deduce the value of the hybrid part H_3 :

$$\begin{aligned}
H_3 &\equiv H_2 \wedge \left(\neg(C_{B,\{m_2\},1} < 0) \vee \neg(\pi_B^{3'} < \pi_B^3 - C_{B,\{m_2\},1} \cdot T_3) \right) \\
&\quad \wedge (\pi_A^3 = 0) \wedge (C_{A,\{m_1\},2} < 0 \wedge (\pi_A^{3'} = \pi_A^3 - C_{A,\{m_1\},2} \cdot T_3)) \\
&\quad \wedge (\pi_B^3 = 1) \wedge \left(\neg(C_{B,\{m_2\},1} > 0) \vee (\pi_B^{3'} > \pi_B^3 - C_{B,\{m_2\},1} \cdot T_3) \right) \\
&\quad \wedge (\pi_B^3 = \pi_B^{2'}) \wedge (\pi_A^{2'} = 1) \\
H_3 &\equiv \left(\neg(C_{B,\emptyset,0} > 0) \vee \neg(1 > \pi_B^{0'} - C_{B,\emptyset,0} \cdot T_1) \right) \\
&\quad \wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = 1 - C_{A,\{m_1,m_3\},1} \cdot T_1) \wedge (\pi_A^{0'} = 0) \\
&\quad \wedge \left(\neg(C_{A,\{m_1\},1} > 0) \vee \neg(1 > \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2) \right) \\
&\quad \wedge \left((C_{A,\emptyset,0} > 0) \vee \neg(C_{A,\{m_1\},1} < 0) \vee \neg(1 < \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2) \right) \\
&\quad \wedge (C_{B,\emptyset,1} < 0) \wedge (1 = 0 - C_{B,\emptyset,1} \cdot T_2) \\
&\quad \wedge \left(\neg(C_{B,\{m_2\},1} < 0) \vee \neg(\pi_B^{3'} < 1 - C_{B,\{m_2\},1} \cdot T_3) \right) \\
&\quad \wedge (C_{A,\{m_1\},2} < 0 \wedge (\pi_A^{3'} = 0 - C_{A,\{m_1\},2} \cdot T_3)) \\
&\quad \wedge \left(\neg(C_{B,\{m_2\},1} > 0) \vee (\pi_B^{3'} > 1 - C_{B,\{m_2\},1} \cdot T_3) \right)
\end{aligned} \tag{28}$$

B.4 Computation of D_4 and H_4

$$\left\{ \begin{array}{c} D_4 \\ H_4 \end{array} \right\} \left(\begin{array}{c} T_4 \\ \top \\ B+ \end{array} \right) \left\{ \begin{array}{c} D_3 \equiv (\eta_A = 2 \wedge \eta_B = 1) \\ H_3 \end{array} \right\}$$

$$D_4 \equiv D_3[\eta_B \setminus \eta_B + 1] \equiv (\eta_A = 2) \wedge (\eta_B + 1 = 1) \equiv (\eta_A = 2) \wedge (\eta_B = 0) \tag{30}$$

$$H_4 \equiv H_3 \wedge \mathcal{F}_B(T_4) \wedge \Phi_B^+(T_4) \wedge \neg \mathcal{W}_B^+ \wedge \mathcal{A}(T_4) \wedge \mathcal{J}_B \tag{31}$$

We calculated the formulas necessary to define $\mathcal{F}_B(T_4)$ and $\neg \mathcal{W}_B^+$:

$$\begin{aligned}
IW_A^- &\equiv (\eta_A > 0) \wedge ((\eta_A = 2) \wedge (m = 1) \wedge \Phi_A^{m_1, m_3} \wedge \Phi_{A-}^{m_1, m_3} \\
&\quad \Rightarrow (C_{A, \{m_1, m_3\}, 2} < 0) \wedge (C_{A, \{m_1, m_3\}, 1} > 0)) \\
&\equiv \perp \\
IW_A^+ &\equiv (\eta_A < b_A) \wedge [\dots] \equiv \perp \\
IW_B^+ &\equiv (\eta_B < b_B) \wedge ((\eta_B = 0) \wedge (m = 1) \wedge \Phi_B^{m_2} \wedge \Phi_{B+}^{m_2} \\
&\quad \Rightarrow C_{B, \{m_2\}, 0} > 0 \wedge C_{B, \{m_2\}, 1} < 0) \\
&\equiv \perp \\
EW_A^- &\equiv (\eta_A = 0) \wedge [\dots] \equiv \perp \\
EW_A^+ &\equiv (\eta_A = b_A) \wedge (\Phi_A^{m_1, m_3} \Rightarrow C_{A, \{m_1, m_3\}, 2} > 0) \\
EW_B^+ &\equiv (\eta_B = b_B) \wedge [\dots] \equiv \perp
\end{aligned}$$

With these results, we deduce that $\neg \mathcal{W}_B^+ \equiv \neg IW_B^+ \wedge \neg EW_B^+ \equiv \top$.

– Computation of $\mathcal{F}_B(T_4)$:

$$\begin{aligned}
\mathcal{F}_B(T_4) &\equiv ((\eta_A = 2) \wedge \Phi_A^{m_1, m_3} \wedge (C_{A, \{m_1, m_3\}, 2} > 0) \\
&\quad \wedge (\pi_A^{4'} > \pi_A^4 - C_{A, \{m_1, m_3\}, 2} \cdot T_4)) \Rightarrow \mathcal{W}_A^+ \\
&\quad \wedge ((\eta_A = 2) \wedge \Phi_A^{m_1, m_3} \wedge (C_{A, \{m_1, m_3\}, 2} < 0) \\
&\quad \wedge (\pi_A^{4'} < \pi_A^4 - C_{A, \{m_1, m_3\}, 2} \cdot T_4)) \Rightarrow \mathcal{W}_A^-
\end{aligned} \tag{32}$$

We deduce that $\mathcal{F}_B(T_4) \equiv (\neg(C_{A, \{m_1, m_3\}, 2} < 0) \vee \neg(\pi_A^{4'} < \pi_A^4 - C_{A, \{m_1, m_3\}, 2} \cdot T_4))$.

– Computation of $\Phi_B^+(T_4)$:

$$\begin{aligned}
\Phi_B^+(T_4) &\equiv (\pi_B^4 = 1) \wedge (\Phi_B^{m_2} \wedge (\eta_B = 0) \\
&\quad \Rightarrow (C_{B, \{m_2\}, 0} > 0) \wedge (\pi_B^{4'} = \pi_B^4 - C_{B, \{m_2\}, 0} \cdot T_4))
\end{aligned} \tag{33}$$

Because $\Phi_B^{m_2} \equiv \top$ and $(\eta_B = 0) \equiv \top$, we deduce:

$$\Phi_B^+(T_4) \equiv (\pi_B^4 = 1) \wedge (C_{B, \{m_2\}, 0} > 0) \wedge (\pi_B^{4'} = \pi_B^4 - C_{B, \{m_2\}, 0} \cdot T_4)$$

– Computation of \mathcal{J}_B :

$$\mathcal{J}_B \equiv (\pi_A^4 = \pi_A^{3'}) \wedge (\pi_B^4 = 1 - \pi_B^{3'}) \tag{34}$$

– For this particular path, $\mathcal{A}(T_4) \equiv \top$.

We can deduce the value of the hybrid part H_4 :

$$\begin{aligned}
H_4 \equiv & H_3 \wedge (\neg(C_{A,\{m_1,m_3\},2} < 0) \vee \neg(\pi_A^{4'} < \pi_A^4 - C_{A,\{m_1,m_3\},2} \cdot T_4)) \\
& \wedge (\pi_B^4 = 1) \wedge (C_{B,\{m_2\},0} > 0) \wedge (\pi_B^{4'} = \pi_B^4 - C_{B,\{m_2\},0} \cdot T_4) \\
& \wedge (\pi_A^4 = \pi_A^{3'}) \wedge (\pi_B^{3'} = 0)
\end{aligned} \tag{35}$$

$$\begin{aligned}
H_4 \equiv & \left(\neg(C_{B,\emptyset,0} > 0) \vee \neg(1 > \pi_B^{0'} - C_{B,\emptyset,0} \cdot T_1) \right) \\
& \wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = 1 - C_{A,\{m_1,m_3\},1} \cdot T_1) \wedge (\pi_A^{0'} = 0) \\
& \wedge (\neg(C_{A,\{m_1\},1} > 0) \vee \neg(1 > \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2)) \\
& \wedge \left((C_{A,\emptyset,0} > 0) \vee \neg(C_{A,\{m_1\},1} < 0) \vee \neg(1 < \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2) \right) \\
& \wedge (C_{B,\emptyset,1} < 0) \wedge (1 = 0 - C_{B,\emptyset,1} \cdot T_2) \\
& \wedge (\neg(C_{B,\{m_2\},1} < 0) \vee \neg(0 < 1 - C_{B,\{m_2\},1} \cdot T_3)) \\
& \wedge (C_{A,\{m_1\},2} < 0 \wedge (\pi_A^{3'} = 0 - C_{A,\{m_1\},2} \cdot T_3)) \\
& \wedge \left(\neg(C_{B,\{m_2\},1} > 0) \vee (0 > 1 - C_{B,\{m_2\},1} \cdot T_3) \right) \\
& \wedge (\neg(C_{A,\{m_1,m_3\},2} < 0) \vee \neg(\pi_A^{4'} < \pi_A^{3'} - C_{A,\{m_1,m_3\},2} \cdot T_4)) \\
& \wedge (C_{B,\{m_2\},0} > 0) \wedge (\pi_B^{4'} = 1 - C_{B,\{m_2\},0} \cdot T_4)
\end{aligned} \tag{36}$$

B.5 Constraints for limit cycle

Here, we assumed that the path studied depicts the limit cycle. Hence, the pre and postconditions apply to the same hybrid state $h_0 = h_4$, which gives the following final set of constraints:

$$H_F \equiv H_4 \wedge (\pi_A^0 = \pi_A^4) \wedge (\pi_A^{0'} = \pi_A^{4'}) \wedge (\pi_B^0 = \pi_B^4) \wedge (\pi_B^{0'} = \pi_B^{4'}) \tag{37}$$

$$\begin{aligned}
H_F \equiv & \left(\neg(C_{B,\emptyset,0} > 0) \vee \neg(1 > \pi_B^{0'} - C_{B,\emptyset,0} \cdot T_1) \right) \\
& \wedge (C_{A,\{m_1,m_3\},1} > 0) \wedge (\pi_A^{1'} = 1 - C_{A,\{m_1,m_3\},1} \cdot T_1) \\
& \wedge (\neg(C_{A,\{m_1\},1} > 0) \vee \neg(1 > \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2)) \\
& \wedge \left((C_{A,\emptyset,0} > 0) \vee \neg(C_{A,\{m_1\},1} < 0) \vee \neg(1 < \pi_A^{1'} - C_{A,\{m_1\},1} \cdot T_2) \right) \\
& \wedge (C_{B,\emptyset,1} < 0) \wedge (1 = 0 - C_{B,\emptyset,1} \cdot T_2) \\
& \wedge (\neg(C_{B,\{m_2\},1} < 0) \vee \neg(0 < 1 - C_{B,\{m_2\},1} \cdot T_3)) \\
& \wedge (C_{A,\{m_1\},2} < 0 \wedge (\pi_A^{3'} = 0 - C_{A,\{m_1\},2} \cdot T_3)) \\
& \wedge (\neg(C_{B,\{m_2\},1} > 0) \vee (0 > 1 - C_{B,\{m_2\},1} \cdot T_3)) \\
& \wedge (\neg(C_{A,\{m_1,m_3\},2} < 0) \vee \neg(0 < \pi_A^{3'} - C_{A,\{m_1,m_3\},2} \cdot T_4)) \\
& \wedge (C_{B,\{m_2\},0} > 0) \wedge (\pi_B^{0'} = 1 - C_{B,\{m_2\},0} \cdot T_4) .
\end{aligned} \tag{38}$$